

An acute severe ehrlichiosis in a dog experimentally infected with a new virulent strain of *Ehrlichia canis*

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INTRODUCTION

Canine monocytic ehrlichiosis (CME) is caused by *Ehrlichia canis*, an obligate intracellular bacterium with tropism for monocytes and macrophages. It is transmitted by the ticks *Rhipicephalus sanguineus* and *Dermacentor variabilis*. CME has been reported throughout the world, with a higher frequency in tropical and subtropical regions. The disease may be manifested by fever, depression, dyspnoea, anorexia, haemorrhages, oedema and weight loss accompanied with laboratory findings of thrombocytopenia, leukopenia, anaemia, and hypergammaglobulinaemia [1–5]. It has been reported that experimental infection of dogs with a cell culture-passaged type strain of *E. canis* Oklahoma induces mild clinical signs and mild pathological alterations in dogs [4]. Therefore, an acute severe experimental canine infection model is needed to study the pathological and immunological pathways induced by virulent *E. canis*. The purpose of the present study was to describe the experimental infection of a dog with a virulent strain of *E. canis* (New Mexico) by analysing haematological and pathological findings.

MATERIALS AND METHODS

Organism and experimental infection of a dog

The New Mexico strain of *E. canis* from a naturally infected dog was maintained through blood transfusion to naïve dogs. A beagle dog was inoculated with a total of 0.5 mL *E. canis* New Mexico-infected canine blood (0.2 mL intradermally and 0.3 mL subcutaneously). Blood samples were collected weekly and clinical alterations were recorded daily.

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PCR

PBLs separation, DNA isolation and PCR were performed as previously described [5]. Total DNA was extracted from canine PBLs (10^7) with the QIAamp blood kit (Qiagen, Valencia, CA, USA) and real-time PCR was carried out to determine levels of *E. canis* DNA based on the 16S rRNA gene in canine PBLs by using the Brilliant[®] SYBR[®] Green QPCR Core Reagent kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions.

Haematology and pathology

Complete blood counts were determined with an automated blood cell counter. The experimental dog was euthanised on day 27 post-inoculation (DPI). Necropsy was performed and macroscopic pathology findings are recorded. The tissue samples from liver, spleen, kidney and lung were collected and processed for histopathology. Paraffin sections were stained with haematoxylin-eosin (HE).

RESULTS

Clinical evaluation and ehrlichemia detection

The dog inoculated with *E. canis* New Mexico developed signs of an acute severe ehrlichiosis such as high fever ($>40^{\circ}\text{C}$), loss of appetite, oedema, dehydration, pale mucous membranes, depression and weight loss, starting from the 19 DPI. A non-steroid anti-inflammatory drug, carprofen (25 mg/day), was orally given to the dog between 22 and 26 DPI. *E. canis* DNA was detected in the peripheral blood leucocytes by real time PCR on the 14 DPI.

Haematology

The significant decrease in thrombocytes ($0.78 \times 10^9/\text{L}$) and moderate decrease in leukocytes ($3.1 \times 10^9/\text{L}$), neutrophils ($2.4 \times 10^9/\text{L}$), lymphocytes ($0.4 \times 10^9/\text{L}$), red blood cells ($3.2 \times 10^{12}/\text{L}$), plasma protein (5.0 g/dL), haematocrit (25%) and haemoglobin (9 g/dL) were determined in the blood sample collected on 21 DPI.

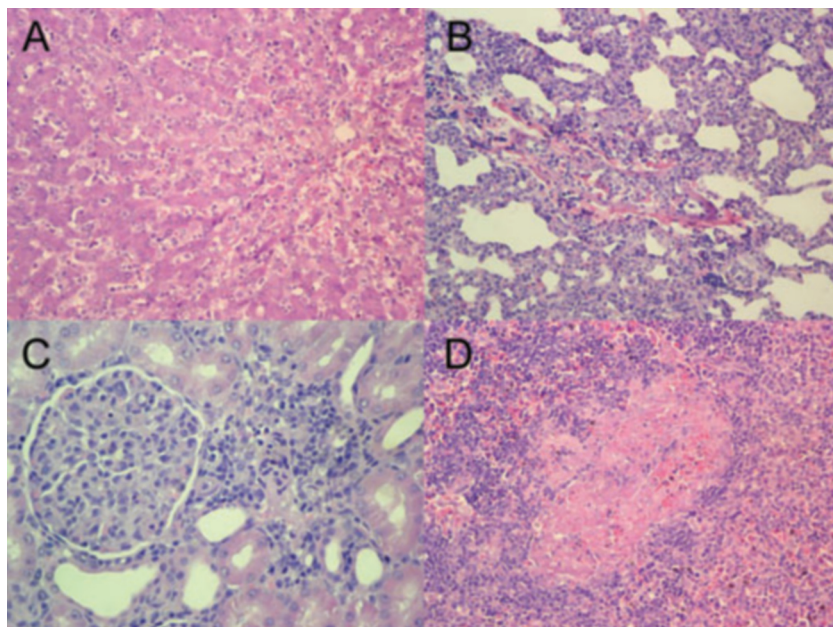


Fig. 1. (a) Liver: severe centrilobular necrosis, sinusoidal dilatation, hyperaemia, mild degeneration in hepatocytes, and plasmocytic-lymphocytic cellular infiltration; H&E $\times 185$. (b) Lungs: prominent mononuclear cellular infiltration in the interalveolar septa and vasculitis; H&E $\times 185$. (c) Kidney: plasmocytic-lymphocytic cellular infiltration, especially in the periglomerular region; H&E $\times 370$. (d) Spleen: necrosis was detected in the germinal centres and a moderate increase in the plasmocytic-lymphocytic cells with hyperaemia; H&E $\times 185$.

Pathology

Gross pathology findings were pale mucous membranes, lymphadenopathy, splenomegaly, ascites, and congestion, petechial and echymotic haemorrhages in the liver, lungs, spleen, heart, lymph nodes and kidneys (Fig. 1). Histopathology of the liver revealed severe centrilobular necrosis, mild degeneration in hepatocytes, plasmocytic-lymphocytic cellular infiltration, sinusoidal dilatation and hyperaemia. Prominent mononuclear cellular infiltration in the interalveolar septa and vasculitis were observed in the lungs. Histopathological findings in the spleen were necrosis in the germinal centres and moderate increase in the plasmocytic-lymphocytic cells with hyperaemia. Plasmocytic-lymphocytic cellular infiltration was observed in the kidney, especially in the periglomerular region.

DISCUSSION

The New Mexico strain of *E. canis* was recently isolated from a naturally infected dog with an acute severe ehrlichiosis [5]. The organism was maintained by serial passage in dogs and the blood from an experimentally infected dog was used for experimental infection of a dog in this study. Establishment of an acute severe ehrlich-

iosis in dogs caused by *E. canis* New Mexico infection may be a useful tool in immunopathological studies of ehrlichiosis.

The pathological findings in various organs were in parallel with previous reports [1,3], such as degeneration, necrosis, lymphadenopathy, vasculitis, haemorrhages, and plasmocytic-lymphocytic cellular infiltration in various organs. These significant pathological findings, especially tissue damage and necrosis, may be initiated with vasculitis and immunosuppression.

In agreement with earlier reports [1,3], the dog demonstrated significant pancytopenia and decreased plasma protein and haemoglobin levels. The alteration of protein metabolism in liver due to tissue damage and bone marrow suppression by the infection may be the reason for these haematological changes. The liver necrosis may decrease the plasma protein level and colloidal osmotic pressure, which may eventually be the reason for oedema in CME. Harrus *et al.* [2] speculated that the mechanism of thrombocytopenia in CME might be platelet consumption, increased splenic sequestration, and decreased platelet lifespan. Possible depleted synthesis of coagulation proteins in the liver due to necrosis may cause increased platelet aggregation, which may contribute to thrombocytopenia.

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